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# Comparision of Bacterial Flora and frequency of Occurance in Water and Clarias gariepinus Raised in Ponds Fertilized With Raw With Poultry Manure

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**Abstract:**

The use of poultry manure and maggot for pond fertilization and fish food respectively, are age-long aquaculture practices to improve productivity. This study investigated the bacterial load, and frequencies of occurrence in fish raised using poultry manure and maggot.

A total of 200 *Clarias gariepinus* (average weight, 150 ± 0.92 g) were each stocked into four earthen ponds (Dimension 20 × 13 $m^2$, each). Ponds A and B were prepared but not fertilized, while Pond C and D were prepared and fertilized with poultry manure. Ponds A and C were fed commercial diet, while ponds B and D were fed maggot at 3% body weight for 6 weeks. Microbial assay of the culture water, skin and gut of experimental fish were carried out to determine the microbial load, species identification and frequency of occurrence. The effect on water quality parameters was investigated.

Results of the assay showed a higher bacterial count in the water sample, skin and gut of fish from C group ($9.30 \times 10^4$, $2.50 \times 10^4$ and $2.11 \times 10^6$ cfu/ml/$cm^2$/g respectively) while the least was recorded in B ($4.43 \times 10^2$, $2.91 \times 10^3$ and $6.4 \times 10^5$ cfu/ml/$cm^2$/g for water sample, skin and gut respectively). *Basillus spp., Staphylococcus saprophyticus, Escherichia coli, Salmonella spp*. and *Micrococcus spp* were isolated from the fish and water, with high frequency of occurrence recorded for *Salmonella spp, Bacillus spp* and *Micrococcus spp* in the skin and guts of C and D groups.

The result of this study revealed increase in the bacterial load in pond water, fish skin and gut as a result of fertilization with poultry manure.

**Keywords:** Bacterial flora; Catfish; Poultry manure; Skin and gut

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# Introduction

The sustainability of fish production will require operating within the lowest minimum cost at the shortest possible rearing time. This is largely dependent on meeting the nutrient requirement of cultured species in ways that are cost effective. Feed cost accounts for at least 50-60% of the total cost of fish production (Gabriel et al., 2007). High cost of a number of conventional feed ingredients such as fishmeal, soybean meal and maize largely accounts for increasing cost of fish production. The import on aquaculture in sub-Saharan Africa is the low return on investment. Therefore there is the need to explore other forms of food/feed and methods for increasing productivity. Some of these include the utilization of poultry manure to increase primary productivity in fish ponds and the use of maggot as food supplement.

The utilization of poultry manure for the improvement of primary productivity in ponds is well documented. Fresh chicken manure is reported to contain 1.6% nitrogen, 1.5% phosphorus and 0.9% potassium. Fang et al. (1986) reported that 1kg of fish can be produced using about 17 kg of chicken manure. According to Das and Jana (2003), pond fertilization has assumed an important role to supplement nutrient deficiency and augment biological productivity through autotrophic and heterotrophic pathways.

Maggot meal is an animal protein source produced from waste, and has been reported to be highly nutritive with crude protein ranging between 43.9 and 62.4%, lipid 12.5 and 21%, and crude fibre 5.8 and 8.2% (Awoniyi *et al.,* 2003; Fasakin *et al*., 2003a,b; Ajani *et al.,* 2004). According to Fashina– Bombata and Balogun (1997), the cost of harvesting and processing one kilogram of maggot meal is smaller compared to the cost of 1 kg of fish meal, thereby showing the cost effectiveness of using maggot meal in the diet of African catfish.

Though fish come in contact with various kinds of bacteria through water, sediments and food (Rheinheimer, 1985), there is however a growing concern on the influence of bacterial composition of fish, especially in the intestine, on the health and growth of the fish (Naim and Ahmed, 2012), which may result from increased microbial load from the use of poultry manure for pond fertilization as well as production and feeding of maggot to cultured fish. Also a number of health concerns are raised because of the culture media for maggot, which are decaying and filthy products (Calvert et al., 1969; Atteh and Adedoyin, 1993). The fear of the release of high concentration of pathogenic microorganisms with the use of poultry waste and maggots in fish culture must be adequately addressed. This study therefore tends to evaluate the effect of feeding maggot and poultry manure on the bacterial load of fish and pond fertilization.

# Materials and Methods

## Experimental site

The experiment was conducted on the Fish farm of the Department of Aquaculture and fisheries Management University of Ibadan, using four earthen ponds (A, B, C and D) with a dimension of 20 × 13 m2 each with an average depth of 1.5 m. Ponds C and D were each fertilized with 11.7 kg of 100% poultry droppings (FAO, 2007) collected in jute bags, while ponds A and B were not fertilized.

## Maggot production

Droppings of poultry (100%) collected in jute bags were placed in a clean and dry container. The container was moistened with water to prevent drying and exposed in the maggotry section of the farm for 2 days to allow for *Musca domestica* adults to lay eggs on them. They were then covered and left for between 3-5 days to allow larva to be fully grown before harvesting.

## Experimental fish and feeding

A total of 200 *Clarias gariepinus* (average weight 150 g) were randomly stocked into each pond. Fish were starved for 2 days before the commencement of feeding.

The larvae of *Musca domestica* were harvested by flotation method; the manure is mixed with water and the larvae and pupae were floated out and collected with a sieve. Collected larvae were washed and fed to fish, at 3% body weight (Fish biomass) twice daily (between 7.00–8.00 hr and 16.00-17.00 hr) for six weeks. Pond A and C were fed commercial diets (40% crude protein), while ponds B and D were fed maggot meal.

## Water quality

The water quality parameters, Temperature and Dissolved Oxygen were measured using a combined digital YSI DO meter (Model 57). pH was measured using an electronic pH meter (Metler Toledo 320 model), while Nitrates, Nitrite and Ammonia were measured using commercial kits (HACH, Loveland co, USA). All measurements were done weekly between 10.00 hr and 12.00 hr. Water samples were collected 20 cm below pond water surface at three fixed locations per ponds into sterile glass bottles (250 ml). Averages for each pond were recorded.

## Sample preparation

Fish and water samples were collected from the four ponds separately into sterilized bottles. All fish randomly selected did not have gross lesions, indicating they were clinically normal. At the end of the feeding trial, bacterial isolates from the culture water, gut and skin of *Clarias gariepinus* were subjected to microbial analysis, to evaluate the difference in microbial load using pour plate method. Bacterial isolates from the skin (1 $cm^2$) and gut (1 g) were aseptically macerated separately and dissolved in 10 ml distilled water, forming stock solutions. The stock solution was serially diluted ten folds. The Nutrient agar (N.A) and MacConkey Agar (MCA) were prepared according to manufacturers' instruction and sterilized in an autoclave for 15 mins at 121°C. This was removed and allowed to cool before it was poured to plate. After setting, 0.1 ml of the stock solution was spread on already prepared plate in duplicate and incubated for 24-48 hrs. at 37°C.

## Bacterial counts and identification

The organisms grew into visible different colonies after 24 hr

and the late growers at 48 h. Bacterial colonies seen were counted and estimated according to Hedges (2002) and results were expressed in Colony Forming Unit per ml or g (CFU/ml or g).

For identification, distinct colonies were further sub-cultured on freshly prepared NA and MCA. The isolates were then identified using morphological studies, gram staining procedures, biochemical and physiological tests (Chessbrough, 2000).

### Statistical analysis

Bacterial counts in experimental samples were subjected to analysis of variance (ANOVA), where $P<0.05$ was judged indicative of a significant difference. Means were separated using Duncan's Multiple Range Test (Duncan, 1955).

## Results

The result of the bacterial count in water samples, fish skin and guts are presented in table 1. The bacteria detected in the pond water of groups A and B were significantly lower ($P<0.05$) than the numbers detected in ponds fertilized with poultry manure. High indices of $10^4$ were recorded in the culture water of fish fed commercial diets and fertilized with poultry manure. Average bacterial count in the skin ranged between $2.91 \times 10^3$ cfu/cm$^2$ in B and $2.5 \times 10^7$ cfu/cm$^2$ in group C.

The bacterial count in the guts of experimental fish showed significant variations ($P<0.05$), with higher indices recorded in groups C and D. In all, the highest microbial load was observed in the skin of group D.

Table 2 shows the frequency of occurrence of isolated species in fish skin and guts. Percentage occurrence of bacterial flora in fish skin ranged from 2.6 to 10.5%, with the highest occurrence observed for *Bacillus sp*. in group D. The least percentage of occurrences was however recorded in all species observed in groups A and B. For the guts analysis, the occurrence of *E. coli* was highest (10.0%) in groups C and D, while *Bacillus sp*. recorded the same value in group D. *Bacillus sp*. was however not observed in the gut of fish in Pond A, despite the occurrence on the skin.

Pond fertilization caused a significant reduction in the DO levels of ponds as shown in table 4. pH values ranged between 6.91 and 7.15. Total ammonia nitrogen was significantly higher in groups C and D. Similarly turbidity was significantly increased, while there was also a build-up in ammonia, nitrate and nitrite levels as a result of fertilization.

## Discussion

Bacterial load in water were higher for fertilized ponds, with highest indices of $10^4$ CFU/ml reported in this study. This agrees with the work of Omojowo and Omojola (2013), who observed higher microbial load in ponds treated with raw untreated poultry waste.

Similarly, bacterial counts on the skin of fish in ponds fertilized with poultry manure were higher. It has been reported that microbial load of fresh water fish varies depending on the water conditions. The bacterial load in ponds fed commercial diets were significantly higher ($P<0.05$). This may be attributed to uneaten feed waste which is broken down and serve as substrate for microbes. This assertion is supported by Ogbondeminu (1993).

The bacterial loads in guts were more than observed in the skin. This is similar to the reports of Naim (2007), where total bacterial load was highest in the gut when compared to the skin and gills. Similarly, Al-Harbi and Uddin (2005) reported that the intestine of fish is heavily colonized by heterotrophic bacteria higher than observed in the surrounding water. Results of the present study shows that despite the high bacterial count recorded in ponds fertilized with poultry manure, the range ($2.9 \times 10^3$ to $2.5 \times 10^7$ and $3.4 \times 10^5$ to $2.1 \times 10^6$ for skin and gut respectively) falls within the permissible range of $10^2$-$10^7$ (Zmyslowka et al, 2000). Similarly, reports for some freshwater species gave bacterial counts of $10^2$-$10^6$CFU/g (Chytiri et al., 2004; Das and Mukherjee, 1999).

Five bacteria were isolated in all samples with varying frequencies of occurrence, however there is a near balance in the prevalence of gram negative and gram positive bacteria observed in this present study. This is at variance with the work of Al-Harbi and Uddin (2010), where the prevalence of gram negative bacterial was reported in catfish cultured in Saudi Arabia. This variation may be attributed to differences in climatic factors which may have significant influence on the occurrence and survival of organism. The occurrence of the bacteria species identified in this study is in tandem with the bacteria species identified in water used for culture in Nigeria as reported by Okpokwasili and Alapiki (1999). *Salmonella* sp., *Bacillus* sp., *E. coli*, *S. saprophyticus* and *Micrococcus* sp. were more abundant on the skin and in the guts of fish cultured in fertilized ponds. These groups of fish are therefore more susceptible to pathogenic infections (Ampofo and Clerk, 2010). Some of the bacteria identified have been implicated in causing a wide range of infectious diseases such as ophthalamitis,

**Table 1:** Total bacterial count of pond water, fish skin and gut.

| | | | Treatment | |
|---|---|---|---|---|
| **Sample** | **A** | **B** | **C** | **D** |
| Water ( x $10^2$) | $4.95 \pm 1.2^a$ | $4.43 \pm 0.33^a$ | $930.00 \pm 0.57^d$ | $25.00 \pm 0.88^c$ |
| Skin (x $10^3$) | $2.98 \pm 0.33^b$ | $2.91 \pm 9.83^a$ | $25000.00 \pm 0.57^d$ | $6000.00 \pm 0.33^c$ |
| Gut ( x $10^5$) | $6.53 \pm 1.20^b$ | $6.40 \pm 0.88^a$ | $21.1 \pm 0.57^c$ | $34.3 \pm 0.57^d$ |

Means of values with same superscript along rows are not significantly different ($P>0.05$)

**Table 2:** Frequency of occurrence of bacterial species isolated from the skin and gut of experimental fish.

| Pond | Isolate | Skin | | Gut | |
|---|---|---|---|---|---|
| | | Number | % | Number | |
| **A** | *Bacillus spp.* | 1 | 2.6 | 0 | |
| | *S. saprophyticus* | 1 | 2.6 | 2 | |
| | *E. coli* | 1 | 2.6 | 1 | |
| | *Salmonella spp* | 1 | 2.6 | 1 | |
| | *Micrococcus spp.* | 2 | 5.3 | 1 | |
| **B** | *Bacillus spp.* | 1 | 2.6 | 1 | |
| | *S. saprophyticus* | 1 | 2.6 | 2 | |
| | *E. coli* | 2 | 5.3 | 1 | |
| | *Salmonella spp* | 1 | 2.6 | 2 | |
| | *Micrococcus spp.* | 1 | 2.6 | 1 | 2.0 |
| **C** | *Bacillus spp.* | 3 | 7.9 | 4 | 8.0 |
| | *S. saprophyticus* | 2 | 5.3 | 3 | 6.0 |
| | *E. coli* | 3 | 7.9 | 5 | 10.0 |
| | *Salmonella spp* | 2 | 5.3 | 4 | 8.0 |
| | *Micrococcus spp.* | 2 | 5.3 | 2 | 4.0 |
| **D** | *Bacillus spp.* | 4 | 10.5 | 5 | 10.0 |
| | *S. saprophyticus* | 3 | 7.9 | 4 | 8.0 |
| | *E. coli* | 2 | 5.3 | 5 | 10.0 |
| | *Salmonella spp* | 2 | 5.3 | 3 | 6.0 |
| | *Micrococcus spp.* | 3 | 7.9 | 3 | 6.0 |

ear infections, meningitis, wound and food borne infections (Morales *et al*, 2004) in human, when loads are beyond tolerable ranges. *S. saprophyticus* has been demonstrated to cause urinary tract infections (UTIs) in women, while *Salmonella* sp. has been reported to cause enteritis and systematic disease (Shinkafi and Ukwaja, 2010). Adams *et al*., 1999) has demonstrated that fish and fish products are only occasionally associated with Salmonella and that filter feeding shell fish harvested from polluted water have been identified as higher risk products.

Ammonia, nitrate and nitrite levels were significantly higher in C and D groups. This same trend was observed in turbidity. The increase may be considered an index of environmental stress (Chakarabarti and Jana, 1998).

This study has revealed increased occurrence of bacteria flora as a result of pond fertilization using poultry manure. However, the bacterial count resulting from this falls within the tolerable range. Therefore there is the need to consciously regulate the application of poultry manure in fish ponds despite its numerous advantages, such that public health will not be compromised.

**Table 3:** Biochemical characterization used to identify the bacterial species isolated.

| Bacterial isolates suspected | Characteristics of colony | Cell micromophology |
|---|---|---|
| *Bacillus spp.* | Creamy white, raised with rough edges | Gram positive rods |
| *S. saprophyticus* | White colonies with streaks of yellow, slightly raised with smooth Edges | Cluster cocci cells |
| *E. coli* | Creamy, slightly raised with smooth Edges | Gram negative bacilli |
| *Salmonella spp* | Colonies are grayish, white, smooth translucent and convex | Gram negative rods |
| *Micrococcus spp.* | Creamy deep yellow, slightly raised with smooth edges | Gram positive cocci |

**Table 4:** Water Quality Parameters.

| | Treatment | | | |
|---|---|---|---|---|
| **Parameter** | **A** | **B** | **C** | **D** |
| Dissolved Oxygen | $6.92 \pm 0.03^{b}$ | $6.89 \pm 0.06^{b}$ | $6.05 \pm 0.40^{a}$ | $6.12 \pm 0.18^{a}$ |
| pH | $7.00 \pm 0.05^{ab}$ | $6.96 \pm 0.06^{a}$ | $7.15 \pm 0.02^{b}$ | $6.91 \pm 0.04^{a}$ |
| Temperature $^{o}C$ | $25.36 \pm 0.03$ | $25.43 \pm 0.29$ | $25.33 \pm 0.08$ | $25.40 \pm 0.30$ |
| Ammonia | $23.56 \pm 0.88^{a}$ | $22.66 \pm 1.20^{a}$ | $26.50 \pm 0.25^{b}$ | $34.03 \pm 0.08^{c}$ |
| Nitrate | $0.05 \pm 0.02^{a}$ | $0.04 \pm 0.00^{a}$ | $0.83 \pm 0.00^{ab}$ | $0.12 \pm 0.00^{b}$ |
| Nitrite | $0.00 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.05 \pm 0.00^{b}$ | $0.09 \pm 0.00^{c}$ |
| Turbidity | $11.00 \pm 0.57^{a}$ | $12.63 \pm 0.68^{b}$ | $13.23 \pm 0.03^{b}$ | $15.30 \pm 0.05^{c}$ |
| Alkalinity | $19.90 \pm 0.05^{ab}$ | $19.36 \pm 0.69^{a}$ | $21.20 \pm 0.60^{b}$ | $35.36 \pm 0.52^{c}$ |
| Hardness | $20.96 \pm 0.98^{ab}$ | $19.73 \pm 0.63^{a}$ | $22.20 \pm 0.10^{b}$ | $29.70 \pm 0.15^{c}$ |

Means of values with same superscript along rows are not significantly different (P>0.05).

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